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**Trypsin 2.5% in HBSS**  
w/o Phenol Red w/o Calcium w/o Magnesium (10X)

**CAT N** : X0920

**Theoretical pH** :  $7.3 \pm 0.3$

**Osmolarity** :  $390 \pm 10\%$

**Colour** : clear, orange-red solution

**Sterility tests** :

- bacteria aerobic-anaerobic
- bacteria strictly anaerobic
- fungi / yeast

**Activity test** : with the L929 cell line

**Storage conditions** :  $-20^{\circ}\text{C}$

Repeated freezing and thawing will reduce enzymatic activity and should be avoided.

**Shelf life** : 24 months

**Composition** : displayed on website and in catalog; also available on request

**Recommended use** :

Use aseptic technique when handling this medium.

For in vitro laboratory use only, not for drug, human or veterinary use.

**Application** :

Trypsin is a porcine pancreas-derived enzyme that is commonly used for the dissociation and disaggregation of anchorage-dependent mammalian cells and tissues. The concentration of trypsin necessary to dislodge cells from their substrate is dependent primarily on the cell type and the age of the culture. Solutions containing 0.25% trypsin are used most commonly.

**Utilisation** :

Cell exposure to trypsin solutions should be as brief as possible, as trypsin can be harmful to the membrane proteins of susceptible cells and can also be taken up by the cells via pinocytosis. Serum helps to reduce these effects because it contains both proteins that inhibit tryptic activity and factors that assist in repairing any enzymatic damage done to the cells.

In serum-free conditions, soybean trypsin inhibitor and refrigerated temperatures can help to reduce these undesirable effects.

**Dilution Instructions for 10X Solutions**

1. Frozen products can either be thawed in a 37°C water bath or overnight at 2 to 8°C.
2. Aseptically transfer 100 ml of 10X trypsin to a sterile one liter container.
3. Add 800 ml of a sterile calcium and magnesium-free salt solution (as listed below) to the container.  
Dulbecco's Phosphate Buffered Saline (DPBS) catalog N° L0615  
Hank's Balanced Salt Solution (HBSS) catalog N° L0611
4. Mix well for several minutes.
5. Determine the pH of a small sample. If necessary, adjust the pH to 7.2-7.8 with 1N HCl or 1N NaOH.
6. Bring the final volume up to 1000 ml with the sterile salt solution and dispense into smaller volumes.

**Methods for use**

1. Frozen products can either be thawed in a 37°C water bath or overnight at to 2 to 8°C.
2. Aspirate the spent medium from the culture vessel and discard.
3. Rinse the monolayer with either a small amount of trypsin or a calcium and magnesium-free salt solution (as listed above), aspirate, and discard.
4. Add enough trypsin solution, prewarmed in a 37°C water bath, to completely cover the cell monolayer.
5. Incubate the flask at 37°C, or for more sensitive cultures, at room temperature or 2 to 8°C.
6. When the trypsinization process is complete, cells will appear rounded upon microscopic examination and the solution in the flask will appear cloudy. Check the flask often to avoid overexposure which can damage the cells.
7. The trypsin should be neutralized either with serum containing medium or trypsin inhibitor. Gently centrifuge the cell suspension and discard the trypsin-containing supernatant.
8. Resuspend the cell pellet with fresh medium and count or culture as desired.

**Indications of deterioration :**

Trypsin solutions should be clear of particulates and flocculent material. Do not use if solution is cloudy or contains precipitate. Other evidence of deterioration may include color change or degradation of physical or performance characteristics.